

## What happens when a protozoan encounters a previously non-interacting bacterium?

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### SUMMARY

To understand how a protozoan and a smaller organism such as a bacterium that have not previously been in contact can establish symbiosis and coevolve, it is necessary to observe these processes from their first encounter. Instead of tracing back the history of known natural symbiosis, we experimentally simulated a first encounter between a protozoan and a bacterium using *Tetrahymena thermophila* and a genetically engineered auxotrophic strain of *Escherichia coli*. By varying the culture condition, we laid out the following four sets of circumstances: (i) both the protozoan and bacterium can grow autonomously, (ii) only the bacterium can grow autonomously (ordinary predator-prey system), (iii) only the protozoan can grow autonomously, (iv) neither the protozoan nor the bacterium can grow autonomously. Herein, we demonstrate that symbiosis was established in circumstances of (iii) (commensalism) and (iv) (mutualism). More precisely, the protozoan as a predator became extinct because of a defensive adaptation of the bacterium in (i) and (ii), the bacterium grew because of a leakage of nutrients from the protozoan in (iii), and both the protozoan and bacterium grew because of the predation of the bacterium and the leakage of nutrients from the protozoan, respectively, in (iv). These experimental symbioses in (iii) and (iv) might enable us to observe the trajectory of their co-evolution to endosymbiosis.